Compliments of the Author

Biochemical Studies of Mercaptan

DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF PURE SCIENCE OF COLUMBIA UNIVERSITY

BY

FREDERIC GROSVENOR GOODRIDGE, B.A., M.D.

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TO MY WIFE.

ACKNOWLEDGMENT.

My thanks are due to Professor William J. Gies, without whose stimulating personality this work could not have been accomplished, and to Professor Charles C. Lieb for his unvarying courtesy and valuable suggestions. F. G. G.

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February 5, 1915.

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CHAPTER I.

Introduction.

In 1833, in the forty-first volume of Schweigger-Seidel's Jahrbuch, Zeise reported the discovery of a new class of sulfur compounds to which he gave the name mercaptan, because of their great affinity for mercury—*corpus mercurio aptum.*[1] In the next year an abstract of the paper by Zeise appeared in the Annalen, with the criticism and praise of Liebig. Since then articles on the pure chemistry of the mercaptans have appeared at rather rare intervals in the various chemical journals. Biochemical studies of the thio-alcohols have also been undertaken, but the research in this field has been desultory and casual. Bacteriologists have noticed the production of mercaptan by anaerobic micro-organisms, and students of hygiene have superficially examined its occurrence in corrupt atmospheres, but the toxic effects of this substance have not been carefully investigated.

In his original communication, Zeise reported the preparation of several sulfur-containing compounds which on further investigation proved to be definitely different from one another. A neutral member of these he named thialol, and another, which possesses many of the peculiarities of sulfo-cyanic acid, he named mercaptan.

Pure ethyl-mercaptan is a colorless, ether-soluble substance, slightly decomposed by light, has a specific gravity at 15° C. of 0.842, and a boiling point, at 28 millimeters mercury, of 61–63° C. It volatilizes at —22° C., and is very inflammable. It is slightly soluble in water, but very soluble in alcohol and ether. The aqueous or alcoholic solutions turn platinum chloride pale yellow, silver nitrate and mercuric oxide white, copper and lead acetate pale yellow, and in the presence of an excess of mercaptan no more metal will remain in the solution. In its action on the oxides, mercaptan shows a noticeable variability. On calcium oxide it has no effect, on copper oxide

[1] Zeise: Liebig's Annalen, 1834, xi, p. 10.

a slow action, on lead oxide an abundant precipitate is formed, while with gold and silver oxides, especially when diluted with alcohol, it causes a marked rise in temperature. With calcium hydroxide in either aqueous or alcoholic solution it has no reaction.

On boiling with potassium hydroxide mercuric mercaptide is not changed. Oxidizing agents do not affect it, except nitric acid, by which it is violently decomposed with the formation of an oily product. By concentrated hydrochloric acid it is dissolved into a clear fluid which is turned pale yellow by the addition of a potassium salt. *Aqua regia* also decomposes it with the formation of sulfur chloride and an unusually pungent steam. Melted mercuric mercaptide is decomposed by equal parts of metallic lead and lead mercaptide, and lead amalgam is formed.

It has been attempted unsuccessfully to join the radical mercaptan to sulfur by fusion. When mixed with mercuric chloride they fuse together easily, and on heating at a high temperature a colorless, thin, ethereal fluid is formed which differs from mercaptan. This contains chloride from which it is separated by decomposition with metallic mercury, leaving a metallic mass as a residue which may be drawn into long ribbons.

Of the lower mercaptans, the methyl compound, CH_3SH, has received the most attention on account of its frequent presence in the animal body. Many of its characteristics are possessed in common with the other lower thio-alcohols, which may be enumerated as follows: Methyl-mercaptan is a fluid with a most unpleasant odor, and a boiling point at about 6° C., and a freezing point at —130.5° C. It is barely soluble in water (5 parts in 1000), easily soluble in alcohol and ether, and in aqueous alkaline solutions with the formation of relatively stable alkaline salts. By means of mild oxidizing substances, as iodine, methyl-mercaptan is changed to methyl sulfide:

$$2CH_3SH + I_2 = (CH_3)_2S_2 + 2HI$$

It enters into combination easily with the heavy metals to form mercaptides. The mercuric compound, $(CH_3S)_2Hg$, is

best obtained by passing methyl-mercaptan through a 3 per cent. solution of mercuric cyanide. The mercuric mercaptide thus formed is a white salt which turns gray on exposure to the air. It consists of ill-defined, four-sided prisms which melt at 175° C., and are insoluble in water, alcohol and ether. With corrosive sublimate, a double union is formed, $(CH_3S)_2Hg.HgCl_2$, from which mercuric mercaptide, $(CH_3S)_2Hg$, may be obtained. The normal mercuric mercaptide goes over into the double salt on the addition of cold concentrated hydrochloric acid. A soluble double salt is formed by the digestion of the insoluble normal mercaptide with a strong mercuric acetate solution. By dry heating, mercuric mercaptide is decomposed into the metal and the disulfide:

$$\begin{matrix} CH_3S \\ \\ CH_3S \end{matrix}\!\!\rangle Hg = CH_3S.SCH_3 + Hg$$

The lead compound is most easily prepared by passing the mercaptan gas into a 3 per cent. solution of lead acetate. This forms a yellow precipitate consisting of plates and prisms which turn brown on exposure to the air. Lead mercaptide is insoluble in water, alcohol and ether and fairly soluble in concentrated solutions of lead salts. Hydrogen sulfide changes the yellow crystals to brown on account of the formation of lead sulfide; the color can be restored, however, by washing with alcohol. On heating the dry lead mercaptide it is changed to lead sulfide and methyl sulfide:

$$(CH_3S)_2Pb = (CH_3)_2S + PbS$$

The mercaptides formed with the salts of the precious metals are generally more soluble than the mercuric or plumbic compounds. The reactions are very delicate and take place easily in the presence of free mineral acids.

History.

This study of the biochemistry of the mercaptans was undertaken as one of a series of investigations of the relationship between the unoxidized sulfur products and certain intoxications.

The further decomposition of the protein molecule by means of the anaerobic bacteria existing normally in the intestinal tract, as *B. lactis aerogenes*, *B. bifidus*, and the *B. coli communis*, gives rise to many substances of both the aromatic and aliphatic series. If these substances enter the blood in the process of absorption they pass through the portal vein into the liver and are there conjugated with acetic, sulfuric, glycuronic or taurocholic acids, etc., synthesized, oxidized or reduced, etc., and pass into the general circulation in a changed condition to be eliminated in the breath, sweat, feces or urine. Many of these substances possess a certain amount of toxicity, but under normal conditions they are so changed as to be relatively harmless to the organism. In disease, however, when the normal defenses of the body are temporarily out of gear or permanently broken down, the metabolic processes are so changed that some of these poisons may reach the tissues and become a menace.

The phenol and indol groups of the aromatic products of putrefaction have been assiduously studied and the presence of indican has been accepted as a measure of the extent of the intestinal putrefaction present. But the toxicity of these aromatic products will result in the appearance of such symptoms as discomfort and headache, which are rather mild. There are certain diseases, however, that suggest a cause associated with faulty intestinal processes, the symptoms of which are convulsions due to an irritation of the cerebral cortex, hemolysis and destruction of tissue, which must be produced by a far more violent poison or poisons. These would seem to be formed in small amounts, rapidly diffused and fulminating in effect, as in gastric tetany, certain idiopathic epilepsies, the convulsive stage of toxemia of pregnancy and the uremic manifestations of nephritis; or of slow formation and cumulative action as in hepatic cirrhosis, arteriosclerosis, pernicious anemia, the early stages of the toxemia of pregnancy and chronic nephritis. The occurrence of mercaptan in these diseases will be dilated upon in a succeeding chapter.

It is conceivable that some of the toxic action may be caused by the close contact of the poisonous material and the terminal

nerve endings in the mucosa of the intestine (plexuses of Auerbach and Meissner), a semi-permeable membrane alone intervening, but if the poison can pass through the membrane it must get into the blood stream; and if it reaches the blood stream, it will, in all likelihood, be eliminated in the urine, unless a decomposition or a resynthesis and a consequent entire change of chemical and physical condition takes place.

The Formation of Mercaptan in the Body.—The mercaptan group in the various secretory and excretory organs of the body is probably derived from the sulfur-containing protein molecule. The exact stages of the protein decomposition, however, are unknown, and the various means whereby mercaptan may be formed have as yet no experimental basis or very weak proofs.

A theory advanced by Abderhalden[1] and others is that the breaking down of cystin, which is a decomposition product of certain proteins, may give rise to this thio-alcohol group. Cystin is di-thio-diamino-dilactic acid:

$$\begin{array}{ccc} & CH_2\text{–}S\text{–}S\text{–}CH_2 & \\ & \cdot \qquad\qquad \cdot & \\ H_2N\text{–}CH & & CH\text{–}NH_2 \\ & \cdot \qquad\qquad \cdot & \\ & COOH \qquad COOH & \end{array}$$

It occurs in some calculi and in certain urinary sediments, and is obtained by hydrolysis, with weak hydrochloric acid, of horn scrapings, human hair, lamb's wool, etc. By reduction with tin and hydrochloric acid, cystin yields cystein, alpha-amino-beta-thio-lactic acid:

$$CH_2SH\text{–}CHNH_2\text{–}COOH$$

This is a very unstable substance. In the air it rapidly oxidizes to cystin. Ferric chloride also oxidizes it with the production of indigo-blue color. If cystin is decarboxylated, an amino thio-alcohol is formed, which may yield ethyl-mercaptan:

[1] C. A. Hilger: Liebig's Annalen, clxxi, p. 208; Karplus: Virchow's Archiv., 1893, cxxxi, p. 210; Konig: Zeit. f. physiol. Chemie, 1892, xvi, p. 525.

$$\begin{array}{l} CH_2SH \\ \cdot \\ CHNH_2 \\ \cdot \\ COOH \end{array} \longrightarrow \begin{array}{l} CH_2SH \\ \cdot \\ CH_2NH_2 \end{array} \longrightarrow \begin{array}{l} CH_2SH \\ \cdot \\ CH_3 \end{array}$$

Of course with lower or higher homologues of cystin, lower or higher homologues of mercaptan might be produced.

A large part of the breaking down of the protein molecule is accomplished by the micro-organisms of the intestinal tract, and it is quite possible by their action that the mercaptan group is directly split off. It has been shown that the eating of certain vegetables causes a marked output of methyl-mercaptan in the urine, but it has not been demonstrated that as a preliminary stage to this change cystin was formed.

It has been suggested that there may be a direct union of methane and hydrogen sulfide in the intestinal tract. This might be accomplished by the intervention of enzymes or bacteria. *In vitro*, however, the paraffins do not unite directly with hydrogen sulfide, but the body has many methods at its disposal which the laboratory worker lacks.

$$CH_4 + H_2S = CH_3SH + H_2$$

In the taurin arrangement there is another possible derivation of mercaptan. Taurin occurs in the bile as taurocholic acid, which is decomposed by hydrochloric acid to taurin and cholic acid.

$$\underset{\text{(taurocholic acid)}}{C_{26}H_{45}NO_7S} = \underset{\text{(taurin)}}{C_2H_7NSO_3} + \underset{\text{(cholic acid)}}{C_{24}H_{40}O_5}$$

Taurin contains the amino and the sulfonic acid groups, and it is, therefore, both a base and an acid. By the reduction of a sulfonic acid there may be produced, first sulfinic acid and then a mercaptan:

$$\begin{array}{l} CH_2NH_2 \\ \cdot \\ CH_2SO_2OH \\ \text{(taurin—a sulfonic acid)} \end{array} \longrightarrow \begin{array}{l} CH_2NH_2 \\ \cdot \\ CH_2SOOH \\ \text{(sulfinic acid)} \end{array} \longrightarrow \begin{array}{l} CH_2NH_2 \\ \cdot \\ CH_2SH \\ \text{(amino mercaptan)} \end{array} \longrightarrow \begin{array}{l} CH_3 \\ \cdot \\ CH_2SH \\ \text{(mercaptan)} \end{array}$$

Detection and Determination of Mercaptan.—Neither qualitative nor quantitative methods for the determination of organic compounds are as delicate or as accurate, as a rule, as the methods followed in the analysis of inorganic substances. The possible isomeres, the facility with which organic compounds oxidize, reduce and polymerize are among the reasons for the lack of accuracy in many phases of organic analysis. Even in the preparation of compounds from pure material, a yield of about 50 per cent. is in certain cases considered satisfactory. This is especially true of the mercaptans on account of their great volatility. They have, however, an advantage over other substances in that their extremely unpleasant odor facilitates their detection in most minute traces. Fischer and Penzoldt[1] waved a cloth wet with different amounts of ethyl-mercaptan about a lecture hall and found that the olfactories of the students were able to detect 1/460,000,000 of a milligram of the substance. This will give some idea of the extremely powerful odor of the material, but of its disagreeable character only its investigators can have a proper conception. This odor, however, serves as a method for the qualitative determination of the presence of the lower mercaptans, that is more delicate than the spectral analysis of sodium by which only 1/1,400,000 gram of that substance can be detected.

An alkaline solution of sodium nitroprusside[2] colors all mercaptans violet, a color which disappears on acidulating and reappears after the addition of an alkali. The presence of hydrogen sulfide does not interfere with the reaction if an alkaline lead acetate solution is added to the nitroprusside solution, so that the hydrogen sulfide will unite with the lead to form plumbic sulfide.

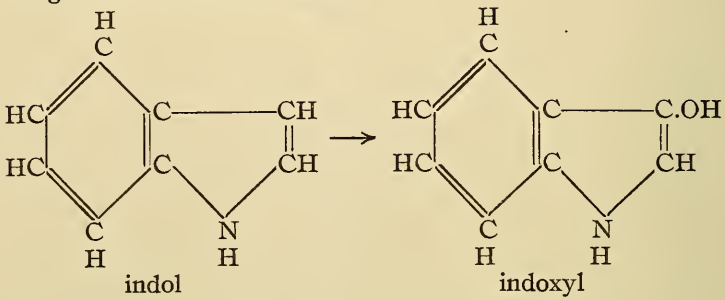
The most serviceable laboratory test for the presence of mercaptan is that dependent upon the change of the color of isatin in concentrated sulfuric acid.[3] Isatin is formed by the

[1] Fischer and Penzoldt: Maly's Jahresbericht der Thierchem., 1886, p. 324.

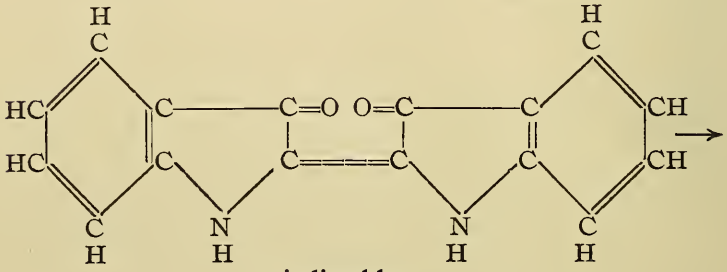
[2] Denigès: Compt. rend., 1889, cix, ——.

[3] C. A. Herter: Jour. Biol. Chem., 1906, i. p. 421. See Denigès, l. c.

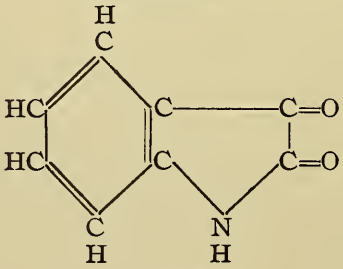
complete oxidation of indol and consists of shining red plates. The following graphic formulae will show the stages of change from indole to isatin:



indol indoxyl



indigo-blue



isatin

The addition of a very small amount of this substance to concentrated sulfuric acid forms a yellow-red solution. In the presence of mercaptan this color is changed at once to a deep green, the color resembling that of bile. Herter found that 25 milligrams of mercaptan changed 50 cc. of

the reddish isatin solution to green in ten minutes. Other sulfur compounds and alcohol, acetone, etc., do not effect the reaction.

For the quantitative determination of mercaptan both colorimetric and gravimetric methods have been used.

Niemann[1] in his study of the separation of carbon dioxide, mercaptan and hydrogen sulfide, from various animal and vegetable food stuffs, employed the following procedure: 500 grams of the substance to be examined are macerated and placed in a three-liter flask to which are added 1000 cc. of distilled water. The contents are then distilled through a Liebig condenser and the vapor collected in a flask containing a 3 per cent. solution of mercuric cyanide. The white precipitate is separated on a hard filter paper, and washed into a flask containing dilute acetic acid. Upon the addition of lead acetate a lemon-yellow precipitate of lead mercaptide forms which is filtered off, dried at 45° C. and weighed. The details of this method were later modified so that the precipitate is placed in a flask about half filled with 10 per cent. acetic acid to which 200 cc. of 3 per cent. lead acetate solution is added through a dropping funnel and 25 cc. of 5 per cent. hydrochloric acid. If the lead mercaptide fails to separate, the solution is heated slowly.

Only one titrimetric method has been described. After precipitation with lead acetate, 25 cc. of 5 per cent. hydrochloric acid are added. The mercaptan gas formed is collected by an outgoing tube into a known amount of decinormal iodine solution. In order to make certain that the gas has completely passed into the iodine solution the flask containing the lead acetate and the hydrochloric acid is gently heated, and the excess of iodine solution is determined by titrating back with standardized sodium thiosulfate solution.

Occurrence of Mercaptan.—The mercaptans occur quite frequently in the animal and vegetable kingdoms. In

[1] Niemann: Arch. f. Hyg., 1893, xix, p. 117.

human beings methyl-mercaptan is found normally in the urine after partaking of asparagus[1], cauliflower and cabbage,[2] and pathologically in certain diseases, as in pneumonia[3]. It is also produced in the ileum and ascending colon by the action of certain of the anaerobic microorganisms on protein food stuffs. L. Nencki[4] found mercaptan constantly present in the gases formed from the decomposition of normal feces. Herter[5], however, failed to find it in the feces, but believed it to be formed higher up in the intestines and to be absorbed. He also concluded that its presence in Nencki's determination was due to the further decomposition of the feces outside of the body, a possibility which Herter avoided by using fresh specimens.

Normal butyl-mercaptan is found in the anal secretion of all the members of the skunk family together with butyl sulfide and traces of methyl-mercaptan.[6] In a report of his travels in northern Texas, Loew says[7]: "I had an opportunity of studying the excretion of *Mephitis Texana*, but the objections of my travelling companions hindered me." He describes the oil as having a very vile garlicky odor. Later Swarts[8] studied the composition of this oil and separated two fractions, one boiling at from 105° to 110° C., and the other at from 190° to 200° C. The oil was very rich in sulfur and contained much of several mercaptans, to which the foul odor was due. Aldrich also found iso-amyl-mercaptan present in the anal secretion of the common skunk.

Upon distillation with steam certain vegetables yield

[1] M. Nencki: Archiv. f. exper. Path. u. Pharmac., **1891**, xxviii, p. 206.

[2] M. Rubner: Arch. f. Hyg., **1893**, xix, p. 136.

[3] J. P. Karplus: Virchow's Archiv., **1893**, cxxxi, p. 210.

[4] L. Nencki: Sitzungsber. d. Mathem. Naturw. Akad. Wien, **1889**, xcvii, part 3, p. 437.

[5] C. A. Herter: Jour. Biol. Chem., **1905**, i, p. 421.

[6] Aldrich: Jour. Exp. Med., **1897**, i, p. 323; Amer. Jour. Physiol., **1901**, v, p. 457.

[7] O. Loew: Aerztl. Intelligenzbl. von München, May, **1879**.

[8] Swarts: Jahresber. f. Chem., **1883**.

slight amounts of methyl-mercaptan. The results of Niemann's[1] researches show these to be:

	Weight. Gm.	Mercaptan. Gm.
Brassica Oleracea Capitata Alba..	500	0.034
" " Botrylis	800	0.168
" " Gummifera.....	800	0.064
" " Cautoropa.....	500	trace
Asparagus.....	...	none
Lettuce	...	"
Spinach.....	...	"
Potatoes	...	"

Semmler[2] also found slight quantities of vinyl-mercaptan in *Allium ursinum.*

Several bacteriologists have found that upon growing certain micro-organisms on special culture media methyl-mercaptan is produced in quite appreciable quantities. In 1899, Nencki and Sieber[3] studied the gases produced by the growth of bacteria on egg white. The *B. liquefaciens magnus* grown under anaerobic conditions on egg white for a period of 13 days produced gases which were analyzed. These were found to be composed of 97.1 per cent. by volume of gases absorbable by potassium hydroxide and 2.63 per cent. hydrogen. Upon dissolving the alkali in water and acidulating with acetic acid a very distinct odor of mercaptan was obtained. The addition of corrosive sublimate or silver nitrate resulted in the production of a white precipitate; while lead acetate and copper sulfate gave yellowish brown precipitates. A year previous to this report Luderitz[4] noticed that, in growing the *B. liquefaciens magnus*, a very vile odor resembling that of decaying cheese and onions was produced.

Karplus[5] found that certain bacteria decomposed the sulfur fraction in the urine and caused the formation of

[1] F. Niemann: Arch. f. Hyg., 1893, xix, p. 117.

[2] F. W. Semmler: Liebig's Annalen, 1887, ccxli, p. 109.

[3] M. Nencki and N. Sieber: Sitsungsber. d. Mathem. Naturwiss. Akad. Wien, 1889, xcviii, part 2b, p. 417.

[4] Luderitz: Zeit. f. Hyg., 1888, v, p. 147.

[5] Karplus: Virchow's Archiv., 1893, cxxxi, p. 210.

small amounts of methyl-mercaptan and hydrogen sulfide. Upon growing the *B. liquefaciens magnus* under anaerobic conditions, Selitienny[1] also found that methyl-mercaptan was formed. Rubner[2] reported the production of the lower thio-alcohol by the growth of *B. proteus vulgaris* in bouillon and gelatine cultures, and also by *B. tetanus* when grown under anaerobic conditions. Metchnikoff[3] failed to obtain any mercaptan from growing cholera bacilli on egg white culture media. Buijwid[4], on the contrary, was quite successful.

Other observers have found that methyl-mercaptan is one of the products of the metabolism of *Penicillium glaucum* and *Saccharomyces cerevissiae*. Mathieu[5] states that ethyl-mercaptan may be formed in any urine which contains sulfur. In the presence of yeast in urine there is first the production of hydrogen sulfide and then of the ethylthio-alcohol. This takes place especially in the early part of the fermentation, when sugar is present in comparatively large quantities.

According to Herter the most active mercaptan producer among the anaerobes is *B. putrificus*. He says: "In every instance in which *B. putrificus* is present in bouillon flasks prepared by growing the mixed fecal flora from cases of intestinal putrefaction there is found also methyl-mercaptan. This observation corresponds to the fact that the *B. putrificus* in pure culture in peptone-bouillon is capable of making mercaptan. It has not always been possible to grow *B. putrificus* from cases in which a methyl-mercaptan reaction was obtained and for this reason, and others, I believe that methyl-mercaptan may be produced by other intestinal organisms than the *B. putrificus*. Nevertheless, in my experience the strongest

[1] Selitienny: Cited after Abderhalden's Biochem.Handlexicon.

[2] Rubner: Hyg. Rundschau, 1893, cxi, p. 525.

[3] E. Metchnikoff: Cited after Abderhalden's Biochem. Handlexicon.

[4] Buijwid: Centralblatt f. Bacteriol., 1893, No. 4.

[5] Mathieu: Bull. de l'Associat. des Chim. de Sucré et Distil., 1911, xxviii, p. 971.

methyl-mercaptan reactions have been obtained from those cases in which *B. putrificus* was present and this bacillus makes more mercaptan when grown in peptone-bouillon than any other anaerobe with which we have experimented. Although not a normal inhabitant, *B. putrificus* owing to its frequent presence in certain foods is usually found in the digestive tract. *B. aerogenes capsulatus* is also a mercaptan producer."

Physiology, Pharmacology and Pathology of Mercaptan.—The principal work on the physiological action of mercaptan has been done by Rekowski[1] in Russia and Herter in this country. The former, working in Nencki's laboratory, experimented on the action of the gas upon white mice and rabbits and the effects of the administration of the calcium compound hypodermically by mouth and by rectum on rabbits. In both groups of experiments the methyl compound was used. Rekowski found that there was almost immediate restlessness; the mucous membranes, the muzzle and the ears became pale and later cyanosed; the pupils were widely dilated; the respirations were 140 per minute, shallow and difficult; there were also involuntary evacuations of urine and feces; paralysis of the hind limbs came on later and was followed by paralysis of the fore limbs and trunk muscles, and a sudden and complete cessation of respiration. In some cases after muscular paralysis appeared, the animal's body was shaken with muscular cramps and it died in opisthotonus.

At autopsy the absence of rigor was noticed as well as the absence of the odor of mercaptan in the tissues. The blood, liver and peritoneum were brick-red. There was rarely hyperaemia or edema of the lungs. Up to one hour after death the auricles were still contracting. The urine usually contained albumin but rarely hemoglobin. The blood contained reduced hemoglobin, which rapidly became oxy-hemoglobin and gave a normal spectrum. Rekowski found the minimal lethal dose for rabbits to be 0.130 gram,

[1] L. Rekowski: Arch. d. Sc. Biol. d. St. Petersbourg, 1893, ii, p. 205.

per kilo of body weight. From this he concludes that mercaptan is considerably less toxic than hydrogen sulfide. A dose of 0.03387 gram, however, caused grave symptoms of intoxication, but the animal recovered at the end of an hour. In the urine the unoxidized sulfur was found to be 40 per cent. of the total sulfur, while usually it is 16.3 per cent.

Herter experimented with ethyl-mercaptan on dogs and monkeys. He failed to obtain any symptoms by injecting on successive days 20, 30, 50, 56, 110, 110, 110, 110, and 110 cc. of a 0.1 per cent. solution into the rectum of a dog of medium size. The injection of 50 cc. of 0.25 per cent. solution was not retained and when 120 cc. of this was introduced into the stomach vomiting occurred. A monkey failed to show any symptoms after the injection per rectum of 10 to 30 cc. of a 0.1 per cent. solution.

Richardson, an English observer,[1] was the first to experiment on the toxicology of mercaptan and like many pioneers was enthusiastic about the possibilities of his subject. He says: "In studying further the disturbances of natural zymosis by secondary chemical products possibly of peripheral or intestinal origin within the body as the result of an imperfect or perverted animal chemistry, I lighted upon some remarkable facts arising from the action of the sulfuretted organic compounds and especially from the compound generally known by chemists under the name of mercaptan or sulfur alcohol.

"The odor which is unmistakable emanates from the skin and breath in those afflicted with dyspepsia, typhus, alcoholic gastritis, delirium tremens and small pox.

"On the blood mercaptan has no effect but when inhaled produces drowsiness, muscular fatigue, anaesthesia and nervous and mental depression which are so severe that if the inhalations are continued may end in self destruction."

Richardson concludes that delirium tremens is due to

[1] B. W. Richardson: Aesclepiad, London, 1889, vi, p. 321.

alcohol, the delirium of small pox and typhus to some unknown sulfur product, and the delirium of melancholia to mercaptan.

Herter[1] found that fecal bacteria from certain patients, when grown in a 2 per cent. peptone solution for twenty-four hours at 37° C., produced varying quantities of mercaptan. The amounts obtained may be summarized as follows:

Normal persons	trace
Babies	strong reaction
Constipated persons	strong reaction
Pernicious anemia	very strong reaction
Marasmus	very strong reaction
Depressed mental states	very strong reaction
Fatty diarrhea	trace
Chronic intestinal indigestion	trace

In each of these mercaptan was persistent and not transitory. In the two cases of pernicious anemia studied, the mercaptan disappeared with improvement.

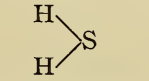
[1] C. A. Herter: "Bacterial Infections of the Digestive Tract," 1907.

CHAPTER II.

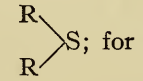
COMPARISON OF THE EFFICIENCY OF VARIOUS METHODS FOR DETECTING AND DETERMINING MERCAPTAN.—INTERFERING FACTORS.—THE VARIOUS MINERAL MERCAPTIDES.—BACTERIOLOGICAL STUDIES.—PHYSIOLOGICAL OCCURRENCE OF MERCAPTAN.—OCCURRENCE OF MERCAPTAN IN DISEASE.

The foregoing résumé of the work that has been done on mercaptan serves to emphasize the possible importance of that substance to the organism, and also shows the incompleteness and lack of continuity of these investigations. Though the presence of methyl-mercaptan has been recognized as one of the constituents of normal urine and a method has been devised for its determination, no authority has ever determined the amount present, and all are contented with the indefiniteness implied by the term "a trace." In regard to its presence in pathological urines the work done has been even less satisfactory.

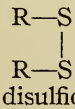
On distillation of urine a number of volatile sulfides are given off. If we represent that organic radical to which the sulfur in the volatile sulfide is attached, by the letter R, we have the following groups of sulfides:



hydrogen sulfide



; for example, ethyl sulfide



disulfide

and R—S—H or mercaptans

These different sulfides seem to be present in varying amounts depending upon the putrefactive process taking place in the organism, and in definite amounts depending upon the "wear and tear" processes in the organism. The

sulfides arising from putrefactive changes are pathological, those due to normal body processes are physiological.

In normal urines sulfides are barely perceptible, whereas in some pathological urines they form a considerable part of the total sulfur content. The mercaptan is closely associated with the other sulfides, and special means have to be used to separate it from the rest, and bring it into a state in which it can be determined.

This investigation, therefore, resolves itself into a double problem—one which has to do with the volatile sulfides in general, and the other which concerns itself specifically with one of their number, mercaptan. The former has been approached only in its relationship to the latter, and offers a fruitful field for future research.

The first experiments were aimed at testing the availability of the different methods for the separation and detection of mercaptan. In these series the method of Nencki and Sieber was used. The following experiments are typical examples of the results obtained from numerous investigations in each series:

Series I. *Dog urine.*—500 cc. of *fresh* dog urine to which oxalic acid had been added were distilled through an ordinary Liebig condenser with rubber connections into a three per cent. solution of mercuric cyanide. A greenish discoloration of the mercuric cyanide solution resulted and later on a light brownish granular precipitate formed. The solution was set aside and the precipitate allowed to settle for twelve hours. During the distillation there was no odor of mercaptan, but a slight odor of hydrocyanic acid from the solution. The possible reactions that may have taken place can be represented as follows, depending upon the sulfide with which the cyanide interacted:

$$(1)\quad 2C_2H_5SH + Hg(CN)_2 = (C_2H_5S)_2Hg + 2HCN$$
$$(2)\quad H_2S + Hg(CN)_2 = HgS + 2HCN$$
$$(3)\quad (C_2H_5)_2S + Hg(CN)_2 = HgS + 2C_2H_5CN$$

The preceding experiment was repeated, but instead of

using fresh urine, old, unpreserved dog urine was used. A black granular precipitate was obtained in the mercuric cyanide solution. The precipitate was considerably heavier than the one which resulted from fresh urine. This increased amount of volatile sulfides probably means that bacterial or other influences have changed the urinary sulfur constituents to the sulfide or mercaptide state. This bacterial action may take place in several ways. It may be a simple reduction of an oxidized sulfur compound, or the special action of certain bacteria, as the *B. disulfuricans*, may have caused sulfide formation.

These experiments were repeated with urines to which thymol or chloroform had been added as a preservative agent. These substances act very efficiently, for even after a week's standing, the urines behaved, as far as the volatile sulfides go, like freshly voided dog urine.

Series II. *Human urine.*—About one liter of freshly voided human urine was treated with oxalic acid and distilled through a Liebig condenser, and the vapor was collected in 75 cc. of a 3 per cent. solution of mercuric cyanide. A very slight, brownish yellow precipitate formed in the mercuric cyanide solution which turned a greenish tinge. This experiment was repeated with urine that had been long standing unpreserved, as well as with urines that had been preserved. The yield of volatile sulfides obtained from the distillation of human urine appears to be not more than one-sixth as great as that obtained from dog urine.

It was realized that very small amounts of the sought-for substances were present in any case, and it was feared that the sulfur constituent contained in the rubber connections of the condenser, etc., might provide a source of error in the ultimate determinations. A special apparatus was designed and constructed which had glass connections throughout. This proved most serviceable and was used in all the subsequent investigations.

Series III. *a.* Two cubic centimeters of ethyl-mer-

captan[1] were added to one liter of distilled water and the whole, with the addition of oxalic acid, was distilled into 100 cc. of a 3 per cent. solution of mercuric cyanide. A heavy, greyish white, waxy precipitate soon appeared and continued to descend for two hours, when the distillation was stopped. During the experiment there was present a very strong odor of mercaptan, showing that the mercuric cyanide did not completely absorb the gas. On microscopical examination the substance precipitated was found to consist of a powder without any characteristic form. On account of the lack of a crystalline form of the mercuric cyanide precipitate which would afford a clue to its identification on direct examination, the following experiments were made in which other salts than those of mercury were used.

b. The experiment was repeated. The distillate was collected in a 3 per cent. solution of lead acetate. A heavy, yellowish precipitate resulted which on filtering and examining microscopically showed the characteristic grayish yellow plates with irregular edges of lead mercaptide.

c. The experiment was again repeated, and the distillate was collected in a solution of bismuth nitrate. A heavy, brownish black precipitate resulted, which on microscopical examination showed very definite small needles of bismuth mercaptide.

d. The substitution of a 3 per cent. solution of ferric chloride for the mercuric cyanide solution did not give any satisfactory results at all. The ferric chloride did not combine well with mercaptan and the amount of the resulting precipitate was very small.

e. When zinc chloride was substituted for mercuric cyanide there was some escape of the mercaptan; but a grayish precipitate formed which showed, under the microscope, the well-defined narrow quadrilaterals of zinc mercaptide.

f. In another series of experiments, gold chloride solution

[1] Ethyl-mercaptan was obtained in 10 cc. quantities in sealed glass tubes.

was used instead of mercuric cyanide. A heavy, yellow precipitate resulted, which on examination with the microscope showed the regular plates and prisms of gold mercaptide.

It was found in all of these experiments that none of the salts used combined with the facility exhibited by mercuric cyanide. But as the quantity of mercaptan present in the urine would under any circumstances be very small, it was thought that the advantage of the ease of detection by means of the definite crystals would counterbalance this lack of combining power. In the use of gold chloride there is theoretically an additional advantage, in that the gold mercaptide formed in the distillation is said to be (according to certain authors) soluble in acetone, whereas all authorities agree as to the general insolubility of the other mercaptides. It was, therefore, hoped that thorough washing of the gold sulfide precipitate with acetone would dissolve out the mercaptide constituent and by means of evaporation and recrystallization a qualitative and quantitative method would be furnished for its detection and estimation.

In spite of numerous attempts, however, with urines to which mercaptan had been added, the gold mercaptide crystals were never obtained by acetone extraction. Gold mercaptide was found to be quite as insoluble in acetone as the other mercaptides.

When urine is distilled directly into solutions of zinc, lead or bismuth salts there is a very heavy carbonate formation which would be hard to get rid of, and would provide a serious source of error in the sulfide determinations.

These substances were, therefore, discarded and the following series of experiments were undertaken with a view to the determination of the availability of mercuric cyanide. Mercuric cyanide was found to be suitable when small amounts of mercaptan were present. When that substance was in concentration equal to a 0.01% solution, however, the mercuric salt could not retain it. As the problem

dealt with small amounts and as no better method could be devised, the mercuric cyanide distillation was retained.

The precipitates were collected, dried for 24 hours at a temperature of 80–90° C. and weighed.[1]

The high temperatures at which the precipitates were dried did not seem to cause any loss through volatilization, for at no time was there the slightest trace of an odor of sulfuretted hydrogen. In order to verify this observation a number of precipitates were dried at a lower temperature and for longer periods. These controls gave approximately the same results for normal urine as the former, so the precipitates from the pathological specimens were dried at the higher temperature.

In order to accomplish the separation of mercaptan from the other sulfides, the following process was employed: The hard filter paper containing the sulfide precipitate was placed in a small flask containing some water. This flask was connected by means of a glass tube with another flask containing 50 cc. of a 3 per cent. lead acetate solution. To the flask containing the precipitate there were added 50 cc. of a 10 per cent. acetic acid solution and 25 cc. of 5 per cent. hydrochloric acid slowly through a thistle tube. The flask was then heated very slightly for an hour, a period during which, if mercaptan is present, the yellow precipitate of lead mercaptide will form in the lead acetate solution, and the crystals may be identified and weighed.

[1] The determinations in the urine are in terms of methyl-mercaptan, as that is the compound generally considered to be present. As the second or mercaptan distillation is made into lead acetate and the substance obtained is lead mercaptide the determination is made by the following proportion:

$$\underset{382}{(C_2H_5S)_2Pb} : \underset{48}{CH_3SH} = \text{wt. of lead mercaptide} : x$$

In the experiments in which ethyl-mercaptan was given directly to a dog the values obtained are in terms of that substance; thus,

$$\underset{382}{(C_2H_5S)_2Pb} : \underset{62}{C_2H_6SH} = \text{wt. of lead mercaptide} : x$$

This was generally the method that was followed and is so far the most reliable means for the quantitative determination of mercaptan. Care should be taken to filter the lead acetate solution through a double filter paper, and to filter the lead acetate plus the lead mercaptide at once after the distillation is completed for fear of a reprecipitation of the lead acetate. As very small amounts of mercaptan are present these precautions are most necessary.

Both the mercuric sulfide and lead mercaptide precipitates should be freed as far as possible from complicating substances by thorough washing with distilled water.

The following series of experiments were aimed at the determination of the mercaptan content in normal human urine, dog urine and human feces. The human urine was collected from students in this laboratory and freshly distilled; that of the dog was from an animal on a general diet; and the feces were procured from hospital patients suffering from slight surgical disturbances which would not be likely to be associated with active protein decomposition or intestinal putrefaction.

Series IV.—The following table will show the amount of volatile sulfides and mercaptan in normal human urine. The determinations of the sulfides are in terms of hydrogen sulfide and those of mercaptan in terms of the methyl-thio-alcohol.

Mercaptan and Volatile Sulfides in Normal Human Urine.

Amount of urine. Cc.	Volatile sulfide. Gm.	Mercaptan. Gm.
1000	0.0028	none
1000	0.0024	none
1000	0.0025	none
1000	0.0029	none
1000	0.0023	none
1000	0.0032	none
1000	0.0028	none
1000	0.0026	none

From the above analyses it will be seen that there is no mercaptan in normal, freshly voided human urine.

The following table will show the figures obtained on the analyses of dog urine:

Mercaptan and Volatile Sulfides in Dog Urine.

Amount. Cc.	Volatile sulfides. Gm.	Mercaptan. Gm.
470	0.0087	trace
450	0.0092	0.0018
480	0.0076	trace
475	0.0079	trace
470	0.0085	0.0014

The volatile sulfides in the twenty-four hour urine of dogs are more than six times the quantity of sulfides present in an equal amount of human urine. Mercaptan is constantly present in dog urine. In the above determinations less than a milligram of mercaptan was considered "a trace." These findings in regard to the volatile sulfides in dog urine are in direct confirmation of the results obtained by Abel.[1]

The feces examined were normally formed human stools. The feces were mixed, unweighed, with a liter of distilled water; oxalic acid was added to acid reaction, and the whole was then distilled as in the urine examination. The fecal distillates differ in appearance from those obtained from urine distillation. The former resemble the precipitate which is formed in passing a stream of hydrogen sulfide into a solution of mercuric cyanide, that is, the precipitates are blackish brown and at once sink to the bottom, whereas the urinary precipitates are grayish brown and at first float near the top of the mercuric cyanide solution. This difference in physical character is doubtless due to the preponderance of hydrogen sulfide in the fecal distillates.

The accompanying table will show the figures obtained for volatile sulfides and mercaptan in normal human feces, freshly passed:

[1] Abel: Johns Hopkins Hosp. Bull., 1894, v, p. 123.

Volatile Sulfides and Mercaptan in Human Feces.

Volatile sulfides. Gm.	Mercaptan.
0.0135	none
0.0120	none
0.0128	none
0.0125	none
0.0167	none
0.0172	none
0.0132	none
0.0134	none

The above experiments, as has been stated before, were with fresh human feces. With feces that had been allowed to stand for a long time before examination, different results were obtained. In one case a jar of feces was allowed to stand for three summer months; upon opening the jar a strong odor of mercaptan was perceptible. The lead mercaptide precipitate was, through oversight, not weighed.

These investigations on human feces bear out Herter's contention that mercaptan is not present in normal fresh specimens of human feces.

The preceding experiments on dog urines were made on animals which received an indefinite diet. The following experiments on dogs were made with controlled diets.

Series V.—A ten-kilogram dog was kept in one of the cages which have long been in constant use in this laboratory.[1] The animal was fed on a rich protein diet, *i. e.*, 48 grams of nitrogen daily. The urines were collected every day and examined for volatile sulfides and mercaptan. The following figures show the results obtained in four consecutive days:

Volatile Sulfides and Mercaptan in the Urine of a Dog Fed on a Rich Protein Diet.

Amount of urine. Cc.	Volatile sulfides. Gm.	Mercaptan. Gm.
455	0.0095	trace
450	0.0090	trace
450	0.0080	trace
445	0.0090	0.0012

[1] Gies: American Jour. Physiol., **1905**, xv, p. 403.

The same dog was then fed for a period of five consecutive days on a poor protein diet, that is, it received 8 grams of nitrogen daily. The urines were then collected and analyzed for volatile sulfides and for mercaptan.

Volatile Sulfides and Mercaptan in the Urine of a Dog Fed on a Poor Protein Diet.

Amount of urine. Cc.	Volatile sulfides. Gm.	Mercaptan.
445	000095	trace
455	0.0089	trace
450	0.0090	trace
450	0.0088	trace
455	0.0092	trace

The volatile sulfides and the mercaptan seem to be uninfluenced by the high or low protein diet. They run a similar course to the larger unoxidized sulfur constituent of which they form a part.

While the methods described for the determination of mercaptan are fair laboratory procedures, still, as it was thought likely that mercaptan may be of clinical pathological significance, attempts were made to improvise a method that would be less cumbersome.

Isatin dissolved in concentrated sulfuric acid forms a red solution which is colored olive-green by mercaptan. The solution of isatin used was made up freshly in each case: 0.05 gram of isatin was dissolved in 1 cc. of concentrated sulfuric acid.

Series VI.—It was found that the common preservatives of urine, such as thymol and chloroform, produce a deep red color, resembling the color of raspberry juice, when added to the isatin solution. In all cases, therefore, it is important to examine the urine when quite fresh, for the use of a preservative is not permissible.

Neither normal dog urine nor human urine when directly added to isatin solution gives a positive reaction. It was also found that upon treating the distillate obtained from dog or human urine with isatin a negative result was ob-

tained. But this does not prove the absence of mercaptan as the following experiments will show:

One liter of water containing one cubic centimeter of a 1 per cent. aqueous solution of ethyl-mercaptan was distilled (after the addition of some oxalic acid) directly into the isatin solution. A negative reaction was obtained. If, however, instead of using 1 cc. of a 1 per cent. ethyl-mercaptan solution, 2 cc. were used, a positive reaction was obtained.

With dog or human urines it was found that with the addition of 2 cc. of a 1 per cent. solution of ethyl-mercaptan to 1000 cc. of urine and then distilling into isatin, a negative result was obtained. In these cases it was found necessary to add 3 cc. of a 1 per cent. aqueous solution of ethyl-mercaptan to a liter of dog or human urine before the distillate gave a positive reaction with the isatin-sulfuric acid solution. From these observations we must conclude that the isatin-sulfuric acid reagent is not sufficiently delicate to employ as a means for the detection of mercaptan in very small quantities. It is a good qualitative test when the amount of mercaptan is large.[1]

It has been observed that iodine solutions oxidize mercaptans to the disulfide state, and it was thought that, perhaps, this reaction may be made available for the determination of the thio-alcohol. The reaction can be expressed by the following equation:

$$2RSH + I_2 = R—S—S—R + 2HI$$

In all the determinations 25 cc. of $N/100$ iodine solution were used, and the loss was determined by titrating with $N/100$ sodium thiosulfate solution after the addition of a few drops of soluble starch as an indicator. Very many experiments were performed with the object of ascertaining whether this method gave reliable results. It was

[1] It must be remembered that other sulfur derivatives, as, for example, thiophene, produce colorations with isatin; and these, if present, will be complicating factors.

found, in general, unreliable, for there are other substances in the urine that will reduce iodine.

Series VII.—The following series of experiments were undertaken in order to determine the amounts of volatile sulfides and mercaptan present in the urine, when the thio-alcohol is administered to the dog *per os*. A dog weighing ten kilos was kept in a cage and fed on a mixed diet of meat, crackermeal, lard, bone ash and water. The dog received daily doses of mercaptan; the urine was collected and analyzed.

Volatile Sulfides and Mercaptan in the Urine of a Dog Fed with Mercaptan.

Dose mercaptan. Gm.	Urine. Cc.	Volatile sulfides. Gm.	Mercaptan. Gm.
0.1	720	0.0134	0.0038
0.15	510	0.0160	0.0040
0.15	535	0.0152	0.0049
0.2	420	0.0188	0.0068
0.15	572	0.0157	0.0044
0.15	505	0.0155	0.0045

No dose of more than two-tenths of a gram was tolerated, and the dog vomited when this dose was repeated. It was found, as will be seen from the above table, that the administration to a dog of mercaptan in the food caused a very marked increase in the volatile sulfides and mercaptan output in the urine, but only a small fraction of the mercaptan administered appeared in the urine as it was eliminated in great part by the breath.

Series VIII. *Bacterial formation of mercaptan.*—The work previously done on the mercaptan-forming capacity of certain bacteria of the intestinal tract has been fairly thorough especially in relation to the *B. putrificus*. The proteus group, however, several members of which are common inmates of the alimentary canal, has been more or less neglected in this connection.

This series of experiments was undertaken with a view to establishing the mercaptan-forming ability of a very common member of the proteus group, *B. proteus vulgaris*.

Cultures of this bacillus grown on a peptone medium were employed. A number of tubes on the style of Einhorn saccharometers were filled in the following manner: Tube number 1 received 10 cc. of the peptone culture of the *B. proteus vulgaris* and 0.1 gram of solid cystin; tube 2, 9 cc. of the peptone culture and 1 cc. of a 0.05 per cent. suspension of cystin in water; tube 3, 8 cc. of the culture and 2 cc. of the cystin; suspension tube 4, 6 cc. of the culture and 4 cc. of the cystin suspension. All the fermentation tubes were incubated at 38° C. In 48 hours there was gas formation in all the tubes except number 1. In 72 hours this also showed the presence of gas in the long arm of the tube. The gas in tubes 2, 3, and 4 smelt strongly of mercaptan. Tube number 1 smelt of hydrogen sulfide rather than of mercaptan.

This experiment was modified. A flask was connected by means of glass tubes so that any escaping gas would be collected in a solution of isatin in sulfuric acid. The flask was filled with 50 cc. of the peptone culture of the proteus vulgaris and 0.1 gram of cystin. At the end of 48 hours in the incubator the isatin-sulfuric acid was tinged with green, and at the end of 72 hours was entirely green.

The above results were also obtained when the flask was kept at room temperature. However, 96 hours were required in this case to change the isatin-sulfuric acid. We must conclude from these experiments that the *B. proteus vulgaris* undoubtedly possesses the faculty of splitting mercaptan from the cystin molecule. This process is not very rapid, however, *in vitro*. The bacillus acts more strongly when the cystin is in solution or in very dilute suspension.

The Occurrence of Mercaptan in Disease.—It has been suggested by one author[1] that mercaptan plays an important role in the depressed or lowered mental states. It was, therefore, thought proper to begin a study of the occurrence of mercaptan in certain forms of insanity. The following results were obtained:

[1] Richardson: Aesclepiad, London, 1889, vi, p. 321.

Volatile Sulfides and Mercaptan in Cases of Insanity.

1. Depressive Mania.

Amount of urine. Cc.	Volatile sulfides. Gm.	Mercaptan.
1230	0.0019	none
1400	0.0028	none
650(?)	0.0016	none
1475	0.0025	none

2. Exalted Mania.

Amount of urine. Cc.	Volatile sulfides. Gm.	Mercaptan.
1420	0.0028	none
1560	0.0032	none
1485	0.0030	none

From these results we see that there is no variation from the normal as far as the mercaptan goes in the cases of dementia studied.

In toxemia of pregnancy owing to the severe disturbances in the metabolism, it was thought likely that some change would be observed in the mercaptan output. We must classify these cases into two types: those that are so severe that they have only a fatal termination, and those cases which are quite mild or recover completely from this disease. The accompanying table will show the results obtained. No variations from the normal were observed in the mercaptan output in these cases. The output of volatile sulfides was increased in two cases.

Volatile Sulfides and Mercaptan in Cases of Mild Toxemia of Pregnancy.

Case.	Amount of urine. Cc.	Volatile sulfides. Gm.	Mercaptan.
1.	1530	0.0042	none
	1510	0.0032	none
	1525	0.0035	none
2.	1260	0.0080	none
	834(?)	0.0040	none
	1255	0.0073	none
3.	1342	0.0072	none

In one severe case, however, which terminated fatally the urine showed distinct traces of mercaptan. The follow-

ing figures will indicate the results obtained on five consecutive days.

Excretion of Mercaptan in Fatal Eclampsia.

Day.	Amount of urine. Cc.	Mercaptan. Gm.
1.	780	0.0022
2.	620	0.0017
3.	685	0.0021
4.	580	0.0024
5.	840	0.0019

In chronic interstitial and chronic parenchymatous nephritis the same results were obtained as in the mild cases of eclampsia. There was no mercaptan in the urine.

Volatile Sulfides and Mercaptan in Chronic Nephritis.

1. Chronic Interstitial Nephritis.

Case.	Amount of urine. Cc.	Volatile sulfides. Gm.	Mercaptan.
1.	1465	0.0085	none
	1520	0.0070	none
	1528	0.0089	none
2.	1380	0.0060	none
	1365	0.0055	none
	1375	0.0064	none
3.	1535	0.0095	none
	1520	0.0086	none
	1545	0.0089	none

2. Chronic Parenchymatous Nephritis.

Case.	Amount of urine. Cc.	Volatile sulfides. Gm.	Mercaptan.
1.	860	0.0120	none
	930	0.0114	none
2.	1034	0.0052	none
	1140	0.0080	none
	1032	0.0086	none

Lobar pneumonia was especially studied for the mercaptan output. Karplus[1] had reported that he had found mercaptan in a case of pneumonia. It was found that

[1] Karplus: Virchow's Archiv., 1893, cxxxvi, p. 210.

there was a difference in the mercaptan output before crisis and after crisis. Pneumonia is a disease which is especially characterized by an increased fibrin content of the blood. In the pathological process of pneumonia this fibrin is deposited in the alveoli of the lungs. When the stage of resolution sets in the fibrin is broken down, absorbed and the products of decomposition are excreted. It was found, as will be observed on examination of the accompanying table, that mercaptan was present in the pneumonic patients after crisis.

Volatile Sulfides and Mercaptan in the Urine of Lobar Pneumonic Patients.

Case.	Stage.	Am. of urine. Cc.	Volatile sulfides. Gm.	Mercaptan. Gm.
1.	Before crisis	1465	0.0048	none
		1470	0.0054	none
		1460	0.0044	none
	After crisis	1320	0.0190	0.0018
		1260	0.0195	0.0024
		1310	0.0225	0.0020
		1395	0.0200	0.0014

Case.	Stage.	Amt. of urine. Cc.	Volatile sulfides. Gm.	Mercaptan.
		1410	0.0125	trace
		1435	0.0162	trace
		1460	0.0154	none
2.	Before crisis	1530	0.0063	none
		1535	0.0073	none
		1537	0.0080	none
	After crisis	1525	0.0192	trace
		1528	0.0184	trace
		1520	0.0176	none

It was thought advisable to make several sulfur partitions in cases of pneumonia, in order to observe the relation of mercaptan to the other sulfur compounds. Three cases were studied. The following table will show the figures obtained:

Sulfur Partitions in Lobar Pneumonia.

Am. of urine.	Case 1.	Case 2.	Case 3.
Amount of urine	1475 cc.	1160 cc.	780 cc.
Total sulfur[1]	1.2875 gm.	2.0556 gm.	1.8749 gm.
Sulfate sulfur	0.45 gm.	0.83 gm.	0.56 gm.
Sulfate sulfur % of total sulfur	34.8	40.4	30.01
Ethereal sulfate S % total sulfur	4.6	8.4	5.7
Neutral S % of total sulfur	12.5	17.4	16.9
Inorganic sulfate S % of total sulfur	82.9	74.2	77.4
Potassium sulfocyanate[2]	0.0108 gm.	0.0114 gm.	0.0064 gm.
Mercaptan	0.0021 gm.	absent	trace
Volatile sulfides	0.014 gm.	0.0163 gm.	0.0324 gm.

In several cases of carcinoma and sarcoma of the intestinal tract, mercaptan was found only in one case which was very advanced and was considered entirely inoperable. The following table explains itself:

Volatile Sulfide and Mercaptan in the Urine of Cases of Malignant Disease.

Diagnosis.	Am. of urine. Cc.	Volatile sulfides. Gm.	Mercaptan.
1. Cancer of pylorus and lesser curvature	1010	0.0285	0.0037
	1220	0.0300	0.0042
	1210	0.0364	0.0040
2. Cancer of pylorus	1540	0.0058	none
	1535	0.0049	none
	1554	0.0058	none
3. Cancer of esophagus	1580	0.0045	none
	1585	0.0040	none
	1595	0.0048	none
4. Sarcoma of small intestine	1655	0.0207	none

[1] The total sulfur was determined by the Benedict method, the various sulfates by the Folin method, the sulfocyanate by the Rupp, Schied and Thiel method.

[2] Expressed in terms of KSCN.

In case 4 the urine contained a large amount of indican. Case 2 was an operable case.

In a case of pyelitis and cystitis the excretion of mercaptan in the urine was especially high. The following table shows the figures obtained on four successive days:

Volatile Sulfides and Mercaptan in the Urine of Pyelitis and Cystitis.

Day.	Am. of urine. Cc.	Volatile sulfides. Gm.	Mercaptan. Gm.
1.	960	0.0145	0.0037
2.	1020	0.0152	0.0028
3.	1250	lost	0.0039
4.	1375	0.0128	0.0035

In the urines of patients suffering with cholelithiasis no mercaptan was ever found. Many of these patients showed an increase in the urinary pigments, and quite frequently bile pigments were present in the urine. But it seems that the biliary constituent of the urine had no effect in increasing the mercaptan output.

With the urines of diabetic patients, peculiar results were produced. It was found that upon distilling a freshly voided specimen of diabetic urine no mercaptan figure could be obtained. If the urine was allowed to stand with a preservative like chloroform, toluene or thymol, and distillation was then made, a negative result was also found. If, however, the urine was allowed to stand for three days without any preservative and was then distilled, a positive mercaptan reaction resulted. It was found that the quantity of mercaptan present in the stale urine was directly proportional to the amount of sugar (glucose) excreted. If the urine was allowed to stand for several days with the addition of a little yeast the mercaptan production was especially marked. The accompanying table will show some of the figures obtained:

Volatile Sulfides and Mercaptan Excretion in the Urine of Diabetic Patients.

Am. of urine. Cc.	Glucose. %.	Volatile sulfides. Gm.	Mercaptan. Gm.	Condition of urine.
2650	2.5	0.0082	0.0	fresh
2275	2.8	0.0092	0.0	fresh
2640	2.4	0.0102	0.0	fresh
2575	2.3	0.0142	0.0025	stale
2445	3.4	0.0135	0.0042	stale
2750	3.9	0.0154	0.0049	stale

Conclusions.—Mercaptan is a compound which may be produced as a result of the chemical processes in the human organism and is always formed as the result of these processes in the dog. It is not found in normal human urine, but is constantly present in that of a dog. Diet in which the sulfur component is not raised to extremes has no effect on the mercaptan content of the urine. Mercaptan bears a close but indefinite relationship to the other volatile sulfides and may be interchangeable with some of these, notably those of the R_2S form, to which it may be oxidized and, as such, excreted. These transformations and its rapid elimination by the breath prevent the recovery of any large amount of mercaptan from the urine. Mercaptan has been found in those diseases where severe decomposition is prevalent. Fatal eclampsia, pneumonia after crisis, ulcerating carcinoma and marked cystitis gave positive reactions for mercaptan.

Occurrence of Mercaptan in the Gastric Contents after Tying of Pylorus.—The stomachs of seven dogs were tied at the pylorus in various ways so as to block off the pyloric exit and induce fermentation and stasis of the gastric contents. No tetanoid manifestations were observed in the animals, but as the putrefaction was marked, it was thought advisable to examine the gastric contents for the presence of mercaptan. Each stomach was placed separately in a securely sealed jar which was connected with another jar containing calcium hydroxide solution and this in turn was connected with another jar in which was mercuric cyanide. The whole apparatus was connected with

a suction pump and the gases from each of these stomachs were drawn through the solutions. These gases were found to be carbon dioxide and hydrogen sulfide. No mercaptan was found in any instance.

Influence of mercaptan on enzymes.

The action of mercaptan upon the common enzymes may, it was thought, be of significance in establishing its causal relationship to certain diseases. In any case, the study of the thio-alcohols would be incomplete without ascertaining their influence upon these unorganized ferments.

The investigations were made upon ptyalin, pepsin and trypsin as types of animal enzymes, malt diastase as a typical vegetable enzyme, milk fermentation as a representative of bacterial action, and the sugar-splitting power of yeast as a type of enzyme action caused by a fungus.

Salivary Amylase.—To 3 cc. of a one per cent. starch suspension in each of five test tubes there were added two cubic centimeters of saliva. To tubes 1, 2, 3, 4, were added, respectively, 1, 2, 3, and 4 drops of propyl-mercaptan. Tube 5 was used as a control. At intervals the various tubes were tested for maltose with the Benedict reagent. It was found that the mercaptan did not influence the salivary amylase.

Malt Diastase.—Similar experiments were carried out with malt diastase. In this case it was also found that the mercaptan did not inhibit the action of the enzyme.

Gastric Protease.—Fairchild's pepsin was used in these experiments. The proteolytic activity was determined by means of Mett tubes.[1] It was found that the propyl-mercaptan did not inhibit the action of the gastric protease on the egg albumin.

Pancreatic Protease.—Similar results were obtained with trypsin, that is, concentrations of propyl-mercaptan up to four drops in 5 cc. of the solution did not inhibit the tryptic digestion.

[1] Frank: Jour. Biol. Chem., 1911, ix, p. 463.

Effect of Propyl-Mercaptan on Yeast Fermentation.—A five per cent. glucose solution was put into a number of Einhorn saccharometers. To the various saccharometers different amounts of mercaptan were added. A control tube to which no mercaptan was added was also used.

A uniform suspension was prepared of the best yeast in distilled water. To each saccharometer I added one cubic centimeter of this suspension. The amount of fermentation was determined by the quantity of carbon dioxide produced in each saccharometer.

It was found that the propyl-mercaptan inhibited the action of the yeast fermentation.

Effect of Mercaptan on the Souring of Milk.—To a number of test tubes containing fresh milk, various amounts of propyl-mercaptan were added, and a few drops of litmus solution. It was found that the tubes containing the propyl-mercaptan behaved similarly to the control tubes, showing that the thio-alcohol did not prevent the growth of the lactic acid bacillus.

CHAPTER III.

Pharmacology.

In their relation to the problem of autointoxication all the products formed from the decomposition of food substances in the intestinal tract are of interest. Certain of these substances, particularly the phenol and indol groups, amino acid derivatives of which are tyrosin and tryptophan, have been thoroughly investigated and the protective methods employed by the body against them, notably conjugation, oxidation and reduction, have been carefully studied.

Very little work has been done, however, on a large number of sulfur-containing substances which are chiefly derived from the thio-amino acid cystin, and practically no work at all on one of its important derivatives, mercaptan.

As cystin forms a large part of the complex protein molecule and as certain of the common bacteria of the intestinal tract have been found to be capable of producing mercaptan from it, the study of mercaptans especially in relation to their toxicology possesses more than a purely academic interest.

These pharmacologic investigations were made on frogs, guinea pigs, and dogs. In the frogs the drug was injected into the anterior lymph-sac, in the guinea pigs subcutaneously, and in the dogs it was administered by mouth in capsules with the food. Only one dog in six was found to be capable of tolerating even the odor of mercaptan, so the investigations on that animal which could yield the most definite results from a human standpoint were necessarily limited.

The studies of the effect of the drug[1] on frogs were made during the winter months when cold-blooded animals are

[1] Unless otherwise stated *ethyl*-mercaptan was used in the experiments, because of the greater ease of procuring and handling it.

naturally torpid but are not more susceptible to drug action. In these animals and in the guinea pigs solutions of the mercaptan in 50 per cent. alcohol were used. Aqueous solutions were at first employed as controls, but as the results were found to be in every way similar, and as ethyl-mercaptan is only slightly soluble in water (1 part in 100), the controls were discarded as unnecessary.

Experiments on frogs

Dose 0.1 to 0.2 milligram per gram weight of frog.

After the injection of the above quantities there were observed the following symptoms: Discomfort from administration, slightly increased respiration, and with the larger doses an odor of mercaptan. These effects passed off rapidly and were followed by a complete recovery.

Dose 0.25 milligram per gram weight of frog.

This dose caused increased respiration and discomfort. The latter symptom was manifested in an incoördinated effort to escape from the wire cage by pushing the head against the sides either at the wires or at the spaces between them, as if the animal's vision were imperfect. After these attempts the frogs squat with half-closed eyes and move only when stimulated. All the reflexes were present, but, with the exception of the toe reflex, all were less marked than usual. In one-half hour or less complete recovery of the frog was observed.

Dose 0.33 milligram per gram weight of frog.

With the administration of this dose the condition of inertia noticed with the preceding dose became more pronounced and deepened into lethargy. The frog lay on its back with all the reflexes suspended, except the toe reflex. Respiration was shallow and scarcely visible and the heart beat was observed with some difficulty. There were occasional spasmodic contractions of the toes; these contractions are more frequent in the hind feet. In a period of something less than one hour recovery set in.

Dose 0.5 milligram per gram weight of frog.

Upon administration of this dose the condition of lethargy noticed with the smaller dose of 0.33 milligram passed into a loss of animation with a complete suspension of all reflexes. The respiration ceased. The heart beat could not be observed. The frog lay limply on the hand in a comatose state. Nevertheless, in a period somewhat under two hours there was complete recovery.

Dose 0.66 milligram per gram weight of frog.

Of the two frogs which received this amount of mercaptan, one recovered and one died. In the one which recovered the symptoms were the same as those observed with the administration of 0.5 milligram per gram weight of frog. But these symptoms were more pronounced.

Lethal dose.

With a dosage of 1 milligram per gram weight of frog death occurred in ninety per cent. of the cases. This appeared to be nearly instantaneous, but the dividing line between the suspended animation following the smaller doses and this real death was so poorly defined that at least four hours should pass before autopsy.

Post-mortem examination.

In all cases in which death followed the administration of the toxic substance, there was noticed a faint exhalation of mercaptan and an absence of *rigor mortis*. Upon pithing the brain no response was obtained. Pithing the spinal cord produced incoördinated muscular contractions. The muscles were highly irritable, but otherwise appeared normal. The abdominal cavity usually contained serous fluid. All the tissues reeked of mercaptan. The heart was about two-thirds its normal size; the ventricle was pale and firm; the auricular ventricular proportion was unchanged; electrical stimulation produced systole; mechanical stimulation was not followed by a contraction. The lungs and the intestines appeared normal.

Several control experiments were made on frogs which after receiving the injection were placed in water at 32° C. This was done as it was feared that the cold might diminish the power of elimination of the frog. The period of recovery was found to be but very slightly shortened in these cases.

Two frogs which had received 0.33 milligram per gram weight of frog were placed in dry jars, A and B. Jar A was connected with a suction pump, while jar B was not. The frog in jar A was able to turn over and recovered its reflexes twenty minutes sooner than the frog in jar B.

These jars, in another series of experiments, were placed in water at 34° C., without, however, affecting the result in the least.

This experiment was again repeated with another variation. The entering air was passed through water in order to avoid the drying of the frogs' skins, and thus retarding or preventing elimination. The ultimate results were unaffected.

Elimination.

Ethyl-mercaptan is probably eliminated through the skin as evidenced by the odor of the exhalations from the animal.

Conclusion.

Mercaptan has a powerful and lasting anaesthetic effect on frogs. A lethal dose killed by depressing the heart. It is difficult to name the condition which follows the administration of the drug. One observer[1] has called it catalepsy.

EXPERIMENTS ON GUINEA PIGS.

Injections of ethyl-mercaptan in 50 per cent. alcohol were made subcutaneously into the guinea pigs.[2]

[1] Richardson: Aesclepiad, London, 1899, p. 321.

[2] It was found that ethyl-mercaptan was soluble in four parts of olive oil.

Dose 1 milligram per 100 gram weight of pig.

After this dose was given there was slight restlessness of short duration probably caused more by the handling than from the effect of the drug. There was a very slight but transient odor of mercaptan from the breath of the animal.

Dose 2 milligrams per 100 gram weight of pig.

From the administration of this dose the results were likewise very mild. One case, however, showed some temperature reduction and a slight dilatation of the pupils.

Dose 3 milligrams per 100 gram weight of pig.

With this dosage the period of restlessness and discomfort became more pronounced. There was a persistent pupillary dilatation and a variable but constantly occurring temperature reduction.

Dose 4 milligrams per 100 gram weight of pig.

After this dose there followed a short period of dullness and indifference to manipulation, suggesting a slight general anaesthetic effect. The reflexes were all present. The pupillary dilatation was marked. There was a slight temperature fall. The respiration was stimulated. The symptoms gradually disappeared and a return to normal condition followed.

Dose 5 milligrams per 100 gram weight of pig.

The weight of the guinea pig was 210 grams. Upon administration of this dose the animal showed immediate discomfort—scratching its nose, moving its head from side to side and resisting handling. A strong odor of mercaptan was noticed from the breath. After ten minutes tremors developed, the corneal reflex disappeared, the pupils were dilated and failed to respond to light, the temperature fell 3° F. and the respirations became very labored. After fifteen minutes, all the reflexes were lost and the guinea pig could not maintain its equilibrium. After twenty minutes the guinea pig seemed to be dead, but the heart continued to

beat feebly. After thirty minutes the heart stopped beating and the guinea pig was obviously dead.

Autopsy: No *rigor mortis* was observed 30 minutes after death. A strong odor of mercaptan was present from all the tissues. The peritoneum was bluish; the veins engorged; the blood of a dark brown color. The heart did not respond to stimulation. All the heart cavities contained blood clots. The thoracic cavity contained brownish serum. Lungs and other organs were unchanged.

Death appeared to be due to asphyxiation from the rapid reduction of the hemoglobin or to direct respiratory paralysis.

In the second experiment, a guinea pig weighing 304 grams was used. Soon after the administration the animal was very uncomfortable. It squealed readily and scratched its nose. A strong odor of mercaptan was noticed from the breath. After ten minutes, the respirations were increased, and the pupils were dilated, showing a slight response to light. After fifteen minutes the temperature fell 1.5° F. No tremors or loss of reflexes were observed. After thirty minutes the pig could not sit upright, and moved unwillingly. The respiration was rapid and shallow. These symptoms gradually wore off, and at the end of two hours the pig was returned to the cage, entirely recovered.

The third pig weighed 492 grams. After the injection of the dose there was noticed prompt discomfort, but not as marked as in the case of the second pig. The respiration was increased and the heart was very rapid and feeble. A strong odor of mercaptan was smelt on the breath. After fifteen minutes, the pupils were dilated but responded to light. Occasional tremors were observed, and the temperaature fell 2.5° F. After twenty minutes there was a loss of equilibrium and the reflexes.

These symptoms gradually disappeared, and at the end of two hours the animal was returned to the cage apparently recovered, but still weak in the hind legs. This

weakness continued but the pig fed well and seemed contented.

Two days later the pig was found dead in the cage.

Autopsy: Cyanosis of the membranes and tongue. No rigidity or odor of mercaptan from the tissues. No evidence of injury was seen. In the abdominal cavity there was a brownish serum; the vessels were engorged, and the blood was dark. The liver appeared normal. The kidneys were hard, congested and the capsule was adherent. Upon microscopical examination, after staining, the typical lesions of acute parenchymatous nephritis were observed. The thoracic cavity also contained a brownish serum. The lungs were congested and edematous. The ventricles of the heart were empty, while the auricles contained a brownish blood clot. Death was probably due either to nephritis, or the edema of the lungs, or the change in the blood.

Pig number 4 weighed 501 grams. After administration of the dose, discomfort was pronounced and immediate. A strong odor of mercaptan was present on the breath. After five minutes, the respiration was shallow and difficult; the heart was rapid and feeble. After ten minutes equilibrium was lost and all the reflexes disappeared. Marked tremors were present and the temperature was reduced 3° F. After fifteen minutes the pig seemed to be dead, but the heart continued to beat feebly. The animal gradually recovered, however, and two hours later was returned to the cage apparently well but feeble. The following day the pig was found dead.

Autopsy: The post-mortem findings were identical with those of pig number 3, except that the odor of the mercaptan was still present in the tissue. Death was probably due to the nephritis or the condition of the blood.

Pig number 5 weighed 320 grams. After the administration of the dose there was observed immediate discomfort. A strong odor of mercaptan was present on the breath. After ten minutes the pupils were dilated but responded to light. The respirations were gasping and the temperature fell 2° F. No

tremors were present. After fifteen minutes the pig lay on its side unable to move voluntarily. All the reflexes were absent. The heart was rapid. Spasmodic contractions of the hind extremities were observed. After thirty minutes the pig was able to sit up with an effort, but it made no attempts to move about. The contractions of the hind legs continued. After forty minutes, the respiration was very shallow, and the heart beat was scarcely felt. The reflexes, however, began to return. After fifty minutes, the pig was able to nose about feebly and resent interference. Animal gradually recovered and was returned to the cage in good condition. No after ill effects were noticed.

Pig number 6 weighed 450 grams. Immediate discomfort was evident after the administration of the dose; the animal scratched its nose and squealed occasionally. After ten minutes, the respirations were shallow and there was a strong mercaptan odor on the breath. There was slight anaesthesia of the cornea; the pupils were dilated but responded to light. The temperature fell 2.2° F. After fifteen minutes the animal lay on its side. The hind legs were paralyzed. There were tremors and involuntary evacuation of feces. After thirty minutes the animal could rise on its forelegs, but dragged its hind legs. The anaesthesia of the cornea was less marked. The breathing was forced, slow and shallow. This pig at no time showed complete loss of reflexes. All the symptoms gradually wore off and the animal was returned to the cage apparently recovered. Five days later, however, the pig was found dead.

Autopsy: There was no rigidity, no odor of mercaptan and no evidence of injury. The mucous membranes and tongue were cyanotic. In the abdominal cavity, the peritoneum was engorged, and it contained a brownish serum. The blood was very dark and venous. The liver was flabby and the gall bladder was full of bile. The stomach was distended with gas. The kidneys were large, swollen

and hard. The renal capsule was adherent. In general, on microscopical examination the typical lesions of acute parenchymatous nephritis were observed. In the thoracic cavity there was present a bloody serous exudate; the lungs were congested. Heart cavities were full of brownish, clotted blood. Death was due either to the nephritis or the blood condition.

Comparison of the toxic effects of methyl-, ethyl-, propyl-, and butyl-mercaptan on guinea pigs.

In order to compare the pharmacological effects of the four lower mercaptans, a guinea pig was placed in each of four two-liter jars, the drug was introduced on cotton, and the jar carefully sealed.

In jar number 1 was placed a guinea pig weighing 295 grams. Approximately 8.0 cc. of methyl-mercaptan were placed on a piece of cotton and introduced into the jar. Within a few seconds the animal rubbed its nose, but showed no other sign of excitement. In twenty seconds the animal fell over and seemed completely anaesthetized. The respirations were gasping in type. One minute after introduction, the pig was removed from the jar. No heart beat and no respiratory movements were noticed. Relaxation was complete.

Autopsy: The tongue was bluish; a slight odor of mercaptan was present. The muscles appeared bluish and cyanotic. The heart was in diastole, and did not respond to stimulation. The blood was dark and venous. The lungs were congested. The kidneys and liver appeared normal. It was believed that death was due to respiratory paralysis.

In jar number 2 a guinea pig was placed weighing 380 grams. 10 cc. of ethyl-mercaptan were introduced on a piece of cotton. Immediately after introduction the animal showed signs of irritation, such as scratching its muzzle, climbing up the sides of the jar, etc. After thirty seconds the animal was completely relaxed, and lay on its side. After one minute, the breathing was slow, irregular and

gasping. In 1 minute and 20 seconds the pig was removed. The relaxation was complete; complete anaesthesia of the cornea. The heart could be felt; slight tremors of the fore legs were present. The respiration, which had ceased, gradually returned, becoming rapid and shallow. Two minutes after removal the pig tried to sit up. In about three minutes after removal it appeared normal. After four minutes it nibbled at some paper and resisted manipulation. Recovery was rapid and complete.

In jar number 3 a pig weighing 260 grams was placed together with 10 cc. of propyl mercaptan. The animal became at once very irritable, as evinced by the scratching of the nose and climbing up the side of the jar. After 10 seconds the respirations became increased. In 15 seconds the respirations were shallow and gasping. After one minute it lay on its side, completely relaxed, gasping, with eyes closed. The animal was removed from the jar in one minute and twenty seconds. The relaxation was complete; there was no heart beat. After 15 minutes, as the pig showed no signs of recovery, a post-mortem examination was made. The membranes and the tongue were cyanosed. Odor of mercaptan present. The muscles were bluish. The heart was still beating, rapid and very feeble. It was thought at that time that the pig might have recovered if it had been let alone. In 45 minutes the heart stopped in diastole. The lungs were congested. The liver and the kidneys were normal. The veins were engorged.

In jar number 4 a guinea pig weighing 480 grams was placed together with 10 cc. of iso-butyl-mercaptan. Immediately after introduction the animal showed signs of discomfort and irritation. In 30 seconds the respirations were gasping and the eyes were closed. The animal fell over in 1 minute. In 1 minute and 20 seconds there were no signs of respiration. The animal was removed from the jar in 2 minutes. There was complete anaesthesia of cornea, and complete relaxation. In two and a half minutes after removal slight twitchings of the hind feet were ob-

served; the heart could be felt with difficulty. After 3 minutes the respiration returned, shallow and gasping. The corneal reflex was present, but feeble; the pupils were widely dilated but reacted feebly to light. The animal gradually recovered.[1]

The general conclusions that may be drawn from these experiments are: The inhalation of the four lower mercaptans produce identical results, and it is safe to assume that the effects produced by their administration in any other way would be the same. The effects are: first, discomfort, then very rapid and complete anaesthesia. Death is caused by respiratory paralysis. If the pig is very promptly removed very rapid recovery ensues.

Elimination: Up to a certain point the drug would seem to be eliminated by the breath. Over and above this amount which might be considered as the point of tissue saturation, excretion is aided by the kidneys. Proof of this is shown by the experiments on the dog. (See below.)

Effects of mercaptan on a dog.

Great difficulty was experienced in obtaining a dog that would tolerate even the odor of the drug. Finally, after five attempts, a ten-kilo dog of the pointer type was procured. The drug was administered once a week in a capsule with the food after a twenty-four hour fast.

Dose of less than 0.1 gram.

A dosage of less than 0.1 gram caused very slight discomfort and a transient odor of mercaptan on the breath. No increased peristalsis was observed. The drug could not be recovered from the urine or the feces.

Dose of 0.1 gram.

This dosage caused slight discomfort, slightly increased

[1] A control experiment was made. A guinea pig was placed in a sealed jar containing no drug. The animal showed no signs of discomfort. The animal was kept in the jar for three hours, and there was no sign of asphyxia, and the pig was removed in good condition. The animal did not defecate or urinate while in the jar.

the peristalsis and caused a strong but fleeting odor of mercaptan on the breath; 0.0038 gram was recovered from the urine. None was present in the feces.

Dose of 0.15 gram.

After this dose was given a strong odor of mercaptan appeared on the breath. Slight discomfort was present; the peristalsis was increased. An average of 0.0048 gram was recovered in the urine, none in the feces. There was a slight polyuria, and a marked trace of albumin.[1] The albumin entirely disappeared in 48 hours, nor could the drug be recovered from the urine after that time.

Dose of 0.2 gram.

This dosage marked the limit of toleration and was only given once without causing vomiting. The administration was followed by slight discomfort, increased peristalsis, a strong odor of mercaptan on the breath, and usually by vomiting. The urine was diminished in quantity, highly colored, and of increased specific gravity. It contained leucocytes and red blood cells, but no casts or shreds. A large amount of albumin was present. It is very interesting to note that a very definite trace of glucose was detected in this urine; 0.0068 gram of the drug was recovered from the urine, but none from the feces.

EFFECT OF MERCAPTAN ON MAN.

As the result of the exposure to and the inhalation of mercaptan by human beings several symptoms were observed that are highly significant. There was a prompt irritation of the conjunctivae with lacrymation and photophobia. The nasal mucous membrane was also irritated, and a profuse discharge from the nose resulted. Either due to the extremely unpleasant odor or to the fact that some mercaptan was swallowed with the saliva, nausea may result. In susceptible individuals this nausea is quite severe, and may be followed by retching and vomiting.

[1] This albumin was distinctly pathological, and should not be confused with the slight traces of albumin usually present in the urine of a dog.

In one case, the subject was so susceptible that as soon as he worked with the mercaptan for half an hour, he began to feel nauseated; he did not vomit, but severe abdominal cramps made him feel very faint; he had a severe attack of diarrhoea. He felt very much relieved as soon as he left the room. This happened three days in succession.

The feeling of mental prostration and lassitude is actual and not imaginary, and there is a strong desire to sleep as evidenced by constant yawning.

Effect of mercaptan on seedlings.

Five medium petri dishes were sown with timothy seeds, using white blotting paper as the ground. The dishes were kept at room temperature.

To dish number 1 was added 1 drop of 1 per cent. aqueous ethyl-mercaptan, with the result that total death followed at the point of application. The same results were obtained with two and with five drops. The tops of the sprouts turned brown, but, except at the point of application, recovered. These plates were kept uncovered all the time, permitting rapid evaporation.

The other plates were kept covered permitting no free entrance and exit of air and moisture. In this case the addition of a few drops of 1 per cent. aqueous ethyl-mercaptan caused browning and general death.

Effect of mercaptan on blood pigments.

It was thought advisable to investigate the action of the thio-alcohols on blood and blood pigments. Very interesting and instructive results were obtained.

Blood was obtained from a dog by a canula in the femoral artery. The blood was defibrinated by vigorous stirring. The experiments were performed on this defibrinated blood.

The blood was diluted about 30 times with distilled water. To this blood there were added 3 drops of propyl-mercaptan. The blood became immediately brownish in color. Later on (five minutes) disintegrative changes set

in. There was a complete hemolysis of the red blood cells, the albumin became coagulated, and, after a few minutes, settled to the bottom of the test tube. The supernatant fluid was clear, brownish red, and upon examination with the spectroscope gave a spectrum similar to that of hematin. With lesser concentrations of propyl-mercaptan the hemolysis was less and there was no precipitation of the proteins of the blood.

Special attention was paid to the spectrum obtained when hemoglobin solutions were treated with mercaptan.[1] The defibrinated blood was diluted with forty parts of distilled water. To this there was added a very dilute solution of mercaptan. The pigment was then examined with the spectroscope, using oxyhemoglobin as a control. A broad, dark band was noticed between D and E, and a narrower, lighter band was present just to the left of D. This somewhat resembles the bands obtained with methemoglobin, but they are not identical. At first it was thought that, perhaps, the mercaptan hemoglobin band was similar to the hydrogen sulfide-hemoglobin spectrum. A specimen of sulf-hemoglobin was, therefore, prepared by passing hydrogen sulfide into a solution of blood, and the spectrum obtained was then compared with the mercaptan spectrum. It was found that it was distinctly different from the mercaptan hemoglobin.

General conclusions.

Mercaptan when given subcutaneously to either cold- or warm-blooded animals has a marked anaesthetic effect. The first result of the administration is irritation, and then follow promptly abolished reflexes and loss of consciousness. Respiration is at first increased and then slowed. The heart is rapid and feeble, and, in warm-blooded animals, the temperature is much reduced, and the color of the blood is changed to a dark brown. If the elimination by means of the breath is not prompt and thorough, the kidneys be-

[1] In all these experiments *propyl*-mercaptan was used.

come impaired, and acute parenchymatous nephritis supervenes. This condition causes death after an interval of from one to five days. When death follows promptly after the administration it is probably due to respiratory depression.

The inhalation of mercaptan causes rapid and overwhelming results. Anaesthesia is complete in less than a minute, and if the animal is not promptly exposed to the air, death follows quickly from respiratory paralysis.

The administration of the drug *per os* causes nausea, vomiting and increased peristalsis. There is irritation and impairment of the kidneys, and these organs are rendered more permeable to the passage of glucose. This damage, as shown by the urinary findings, rapidly passes off and the kidneys return to normal.

Mercaptan is an irritant poison to living tissue. Exposure, however limited, will cause a pronounced and chronic conjunctivitis and an inflammation of the nasal mucous membrane in human beings. To plant life it is also injurious causing local death at the point of contact, and, if evaporation is diminished, general destruction.

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BIOGRAPHICAL.

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